Intracapsular development in the freshwater gastropod *Chilina dombeiana* (Bruguière, 1789) (Gastropoda: Hygrophila: Chilinidae)

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ABSTRACT

Chilina dombeiana (Bruguière, 1789) is a native Chilean species inhabiting freshwater and estuarine environments. In the present study, a series of stages for embryonic development of the embryo is described. The snails lay gelatinous and transparent zig-zag-like string egg masses, ranging between 10 and 130 mm in length. Each egg mass contains a variable number of embryos ranging from 60 to 298 eggs with a mean density of 2.9 · mm^{-2} (± 0.7 SD). Embryos inside egg masses are individually encapsulated and embedded in a jelly matrix. Hatching as crawling juveniles took place after 28 days, indicating that direct development occurs in this species. In contrast to other freshwater pulmonates, the well-developed operculum observed in *C. dombeiana* from the veliger stage, suggest a marine ancestry for this species.

Additional Keywords: Chilinidae, Bio-Bío river, hermaphroditism

INTRODUCTION

Chilinidae is an ancient family of freshwater gastropods endemic to South America (Jarne et al., 2010). The family is monotypic, with *Chilina* including the primitive pulmonate snails described by Gray in 1828 (Brace, 1983). *Chilina dombeiana* (Burguière, 1789) is a native Chilean species that inhabits freshwater and estuarine environments from approximately 35° S to 37° S (Valdovinos, 2006). Although this species is highly abundant in some rivers and lakes, and may play an important ecological role in freshwater ecosystems (Valdovinos et al. 2006), there is little information about the basic biology of this organism.

Species of the order Hygrophila shows simultaneous hermaphroditism and are capable of self-fertilization and/or biparentality through cross-fertilization (Jarne et al., 2010; Nakadera et al., 2014). However, there is no published information on the reproductive behavior and embryonic development of *C. dombeiana*. We describe herein the intracapsular development of individuals of *C. dombeiana* inhabiting a riverine environment in south-central Chile.

MATERIALS AND METHODS

At one site in the Bio-Bío river (36°49′22.43″ S, 73°6′31.41″ W) in Concepción, Chile, 50 individuals of *C. dombeiana* were collected by hand and transported to the Facultad de Ciencias, Universidad Católica de la Ssma. Concepción. In the laboratory, the *C. dombeiana* adults were cultivated in individual plastic boxes (200 ml) filled with freshwater collected from the sample site. Boxes were constantly aerated with an air pump and the temperature was maintained at 18°C. The water inside the boxes was changed every ten days. Once the individuals had laid egg capsules, the relative duration of each developmental stage (in days), a range of total number of egg by egg mass, egg and embryo size, and embryonic development traits were recorded. In order to make these measurements, 20 egg masses from different individuals were haphazardly chosen and photographed every three days under a microscope (10×; Olympus CX31) connected to a tablet computer equipped with a digital camera (Smart Pad 500). Five stages of embryonic development were observed: early embryo, trochophore, early veliger, late veliger, and pre-hatching juvenile. Embryo size was measured with the software Image measure (VMS3.5). In addition, when the egg mass showed a string formation (Figures 1, 2), the total number of embryos per clutch was estimated by counting the number of embryos in a section of the egg mass and extrapolating to the total egg mass size. This was done using a binocular microscope (0.67×; Olympus CX31).

RESULTS AND DISCUSSION

Sexual maturity of the freshwater snail *C. dombeiana* is reached within the first year of the individual's life cycle

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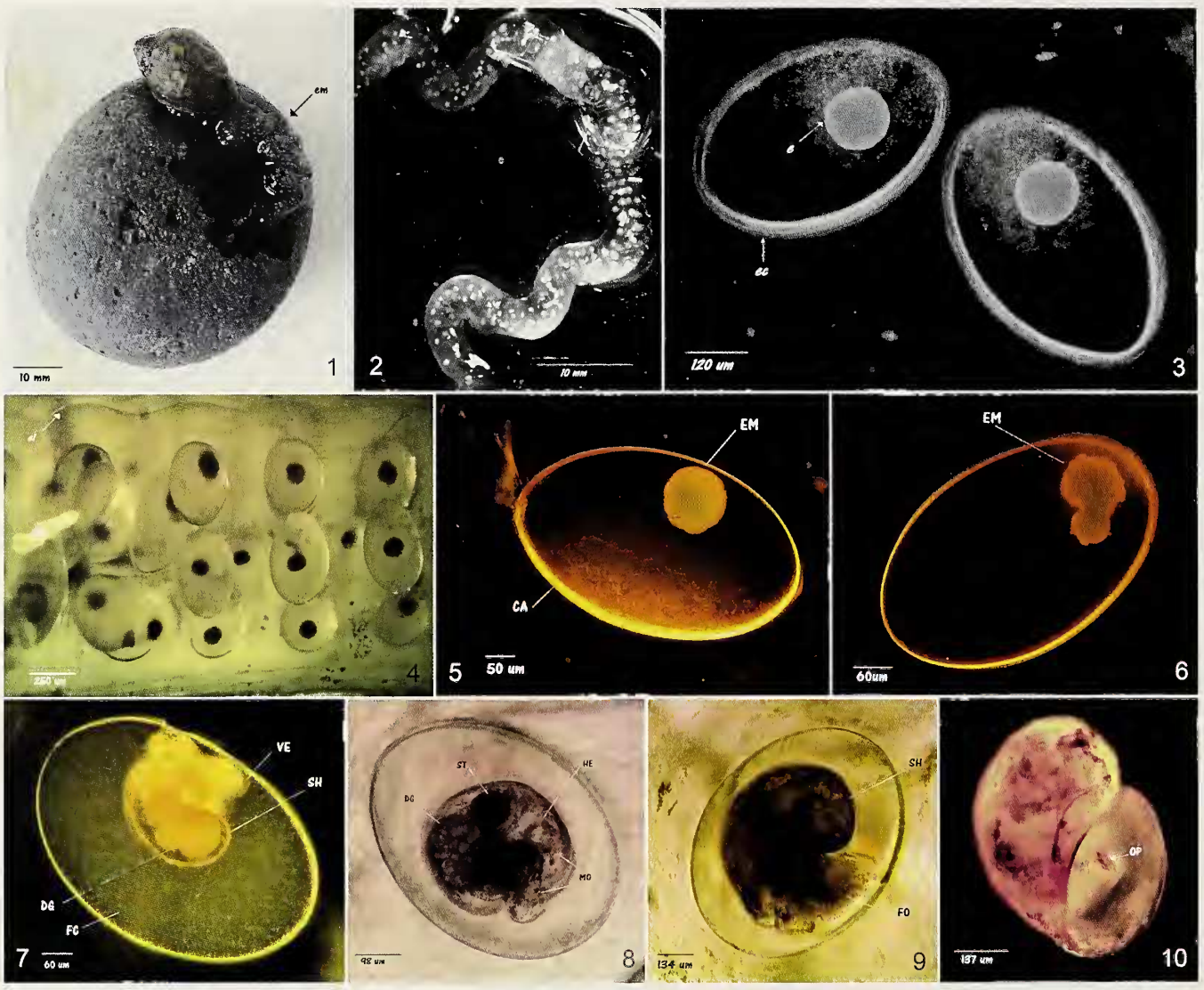

Figures 1–10. *Chilina dombeiana*, egg capsules and intracapsular development. **1–4.** Egg capsules. **1.** Gelatinous egg mass (em) attached to hard substrate. **2.** Egg mass showing the general zig-zag string appearance. **3.** Light micrograph showing the embryos (e) contained within each egg capsule (ec). **4.** Detail of the external layer (el) of the egg mass surrounding the egg capsules. **5–10.** Intracapsular development. **5.** Segmented egg. CA: egg capsule; EM: embryo. **6.** Trochophore stage. EM: embryo. **7.** Early veliger stage. DG: digestive gland; FC: intracapsular fluid; SH: shell, VE: velum. **8.** Veliger stage. DG: digestive gland; HE: heart; MO: eyespot; ST: stomach. **9.** Late veliger stage. FO: foot; SH: shell. **10.** Pre-hatching juvenile. OP: operculum.

(C. Valdovinos; unpublished data). In our observations, the smallest individual laying egg masses was 11.7 mm in shell size. Individuals lay gelatinous and transparent egg masses on rocks.

The egg masses had a zig-zag-like string formation ranging between 10 and 130 mm in length (Figures 1, 2). Within each egg mass, embryos were observed individually encapsulated and embedded within a gelatinous matrix (Figures 3, 4). The total number of embryos per egg mass ranged from 60 to 298 with a mean density of 2.9 eggs · mm^{-2} (± 0.7 SD). In the first 4 days of development, the early embryos were characterized by cleaved eggs and embryos at the pre-trochophore stage with an average size of 120.9 µm (± 11.8 SD; Figure 5; Table 1). The embryos were yellow in color and were embebed in a transparent intracapsular fluid. No aparent major movement of the embryos was noticed. Between days 5 and 6 of cultivation, the embryos developed into early and late trocophores had a mean average diameter of 139.4 µm (± 11.1 SD; Figure 6; Table 1). Between days 12 and 15, early veliger embryos with velum and noticeable development of a very soft shell (average size of 235.5 µm ± 8.1 SD; Table 1) at the apical end were observed (Figure 7). In addition, at this stage, digestive gland and active movement of the larvae inside the capsules were visible. After 16–19 days of development, the intracapsular fluid became more transparent. Embryo at this late veliger stage had a well-developed velum with an average shell size of

Table 1. Developmental time (days) and average size (μm) of embryos of the freshwater gastropod *Chilina dombeiana* at different developmental stages. Thirty embryos were measured at each stage (excepting stage 2 were only 10 embryos were recorded).

Stage	Traits	Mean Size (μm)±SD	Time (days)
1	Egg fertilized with subsequent first cleavages, blastula to gastrula stage.	120.9 ± 11.8	3–4
2	Trochophore stage progresses beeomes to have an elongated shape of embryos	139.4 ± 11.1	1–2
3	Pre-veliger embryos stage displayed movement with noticeable development of very soft shells at the apical end and digestive gland.	235.5 ± 8.1	4–5
4	Veliger embryo stage with the spiralization process of the shell, appearance of two eye spots, heart activity and transparent operculum. Increase calcification of the shell and the development of the digestive gland.	394.5 ± 12.5	10–12
5	Pre-hatching veliger characterized by a hard and dark brown shell, foot and operculum fully developed, and most morphological traits like adults. High mobility of individuals.	505.6 ± 11.4	5–7

394.5 μm (± 12.5 SD) (Figures 8, 9; Table 1). In addition, coiling of the calcified shell, the dark pigmented eyespots, the transparent operculum, and the digestive gland were also evident. At this stage, the embryo had a high heart rate. Between days 23–29, pre-hatching juveniles were observed inside capsules. The shells were well calcified and had a reduced velum (Figure 10). The foot muscle and operculum were fully developed and individuals actively moved inside the capsule. At this stage, the mean shell size was 505.6 μm (± 11.4) (Table 1). Hatching as crawling juveniles took place after 28 days, indicating that direct development occurs in this species.

Adult freshwater pulmonates usually lack an operculum, the exception being the family Amphibolidae (see Golding, Ponder, and Byrne, 2007). *Chilina dombeiana* differs completely from other non-amphibolid freshwater pulmonates by having a well-developed operculum. This suggests that *C. dombeiana* could have an evolutionary origin from marine ancestry. Phylogenetic studies on this group would shed light on the evolution of this species and the potential adaptation of populations of these snails to freshwater habitats (see Harry, 1964; Barker, 2001).

Although the range of *C. dombeiana* is restricted latitudinally from 35° to 37° S (Valdovinos, 2006), this species can be found abundantly at estuarine river mouths all the way to upper river basins characterized by well-oxygenated, clean, and cold water. As has been suggested for other gastropod species (e.g., Pechenik, 1983; 1986; Rawlings, 1996; Pande et al., 2010), the direct and encapsulated development of *C. dombeiana* would allow this organism to tolerate extreme environmental conditions during its entire embryonic phase. Although direct development may lead to restricted dispersal of this species, potentially dislodged egg masses or capsules from the substrata would favor population connectivity and gene flow via river currents. Considering that human use of freshwater ecosystems causes the greatest negative impact on these ecosystems (Strayer and Dudgeon, 2010), large anthropogenic perturbations could expose freshwater invertebrates to specific threats that may result in local extinction processes. Accordingly, Valdovinos (2006) highlighted the vulnerability of the Chilinidae family due to significant habitat lost. In that study, the author further emphasized the importance of conducting more studies that contribute to the understanding of these freshwater gastropods.

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